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(54) Title: NUCLEAR ANTIGEN La

(57) Abstract

A diagnostic test for detection of antinuclear antibody anti-La in a serum sample, comprises contacting the serum sample with a support having immobilised thereon a synthetic polypeptide displaying the antigenicity of all or a porti n f the human autoantigen La, or an antigenically active fragment thereof, and detecting the presence of anti-La antibody bound to the synthetic polypeptide or fragment.

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"NUCLEAR ANTIGEN La"

This invention relates to nuclear antigens, in particular the human autoantigen La, and to the use of a synthetic polypeptide displaying the antigenicity of the human autoantigen La, antigenic fragments thereof or fused polypeptides containing the antigenic polypeptide or fragments in diagnostic tests for the detection of the antinuclear antibody, anti La, in serum.

Autoantibodies reactive with nuclear antigens characterise many human autoimmune diseases (1). The antinuclear antibody (ANA) anti La is very strongly associated with Sjögrens Syndrome, one of the multisystem rheumatic diseases, and serves as a diagnostic marker of that disease (2). The nuclear antigen La, with which anti La reacts, has been ext nsively studied to determine its nature and

possible relationship to the etiology of Sjögrens Syndrome, and, as reported by several groups, La is a protein of MW 48-50kD which associates with a series of small nuclear RNAs (3).

It will be appreciated that polypeptides displaying antigenicity characteristics of the human autoantigen La are of particular utility in highly specific and sensitive diagnostic immunoassays, such as an ELISA for the detection of the antinuclear antibody (ANA), anti-La.

Sensitive immunoassays have previously only been available for the detection of antibodies to autoantigens that are abundant in cells and which can be readily purified biochemically (5,6,7). Biochemical purification of extractable nuclear antigens (ENA) results in extremely small yields of pure autoantigen (8). While the source of tissue for the biochemical purification of La has been bovine or rabbit thymus (9,10,11,12,13), differences have been demonstrated between La from human, bovine and murine tissue (14,15) suggesting that La of human origin would be the preferred source of autoantigen for diagnostic assays.

In accordance with the present invention, there is provided a diagnostic test for the detection of the antinuclear antibody anti-La in a serum sample, which comprises the steps of:

(a) contacting the serum sample with a support having immobilised thereon a synthetic polypeptide displaying the antigenicity of all or a portion of the human autoantigen La or an antigenically active fragment thereof, the amino acid sequence of said polypeptide or fragment comprising or including the sequence

LeuGluGlyGluValGluLysGluAlaLeuLysLysIleIle
GluAspGlnGlnGluSerLeuAsnLysTrpLysSerLysGly
ArgArgPheLysGlyLysGlyLysGlyAsnLysAlaAlaGln
ProGlySerGlyLysGlyLysValGlnPheGlnGlyLysLys
ThrLysPheAlaSerAspAspGluHisAspGluHisAspGlu
AsnGlyAlaThrGlyProValLysArgAlaArgGluGluThr
AspLysGluGluProAlaSerLysGlnGlnLysThrGluAsn
GlyAlaGlyAspGln

or an epitope thereof; and

(b) detecting the presence of anti-La antibody in said serum bound to said synthetic polypeptide or fragment.

This invention also provides a diagnostic test kit for detection of antinuclear antibody anti-La in a serum sample, which comprises:

(a) a support having immobilised thereon a synthetic polypeptide displaying the antigenicity of all or a portion of the human autoantigen La or an antigenically active fragment thereof, the amino acid sequence of said polypeptide or fragment comprising or including the sequence

LeuGluGlyGluValGluLysGluAlaLeuLysLysIleIle
GluAspGlnGlnGluSerLeuAsnLysTrpLysSerLysGly
ArgArgPheLysGlyLysGlyLysGlyAsnLysAlaAlaGln
ProGlySerGlyLysGlyLysValGlnPheGlnGlyLysLys
ThrLysPheAlaSerAspAspGluHisAspGluHisAspGlu
AsnGlyAlaThrGlyProValLysArgAlaArgGluGluThr
AspLysGluGluProAlaSerLysGlnGlnLysThrGluAsn
GlyAlaGlyAspGln

or an epitope thereof; and

(b) means for detecting the presence of anti-La antibody in said serum bound to said synthetic polypeptide or fragment.

A partial cDNA sequence encoding the putative carboxyl-terminal 12% of the human La protein has been published (4), with the prediction that this 55 amino acid region contains an antigenic determinant. Following further investigations in this regard, including the use of probes derived from the published sequence to isolate homologous human cDNA clones which have been used to express portions of the La protein and to map regions of antibody reactivity, significant anomalies have now been shown to exist in the previously published sequence.

In work leading to the present invention, a recombinant DNA molecule has been constructed comprising a nucleotide sequence including all or a portion of the base sequence shown in Figure 1. As described in greater detail below, the nucleotide sequence of Figure 1 is present in a La cDNA clone of 1.4kb, contains an open reading frame of 1.065kb, and encodes a polypeptide of 355 amino acids (which is estimated to be 70-80% of the full length human protein). This work has further revealed that at least one epitope of La is contained in the carboxy-terminal 103 amino acids of the sequence as set out in Figure 1.

This work has enabled the production of fused polyp ptides comprising polypeptide sequences displaying th antigenicity of the human autoantig n

La as the C-terminal sequence, and an additional peptide or polypeptide, for example a polypeptide coded for by the DNA of a cloning vehicle, as the N-terminal sequence fused thereto. Such fused polypeptides can be produced by host cells transformed or infected with a recombinant DNA cloning vehicle comprising an expression control sequence having promoter sequences and initiator sequences, and a nucleotide sequence capable of being expressed as a polypeptide displaying the antigenicity of the human autoantigen La. The fused polypeptide can be subsequently isolated from the host cell to provide the fused polypeptide substantially free of other host cell proteins. If desired, the fused polypeptide may be cleaved to remove the fused N-terminal sequence.

The present invention involves the use of synthetic peptides or polypeptides displaying the antigenicity of the human autoantigen La. As used herein, the term "synthetic" means that the peptides or polypeptides have been produced by chemical or biological means, such as by means of chemical synthesis or by recombinant DNA techniques leading to biological synthesis. Such polypeptides can, of course, be obtained by cleavage of a fused polypeptide as described above and separation of the desired polypeptide from the additional polypeptide coded for by the DNA of the cloning vehicle by methods well known in the art. Alternatively, once the amino acid sequence of the desired polypeptide has been established, for example, by determination of the nucleotide sequence coding for the desired polypeptide, the polypeptide may be produced synthetically, for example by the well-known Merrifield solid-phase synthesis procedure.

Further features of th present invention will be apparent from the detailed description in the following Examples, which are included by way of illustration of the invention.

EXAMPLE 1

This Example describes the identification and characterisation of a human cDNA clone encoding La. In the drawings:

Figure 1 shows the nucleotide sequence of the cDNA of a clone encoding La, together with the amino acid sequence for which it codes.

MATERIALS AND METHODS

Identification of homologous cDNA clones with an oligonucleotide probe.

A cDNA library was constructed in a λgt10 using mRNA from a B cell tumor JOY (16) as described by Hariharan (17). A 37mer oligonucleotide probe was synthesised on an Applied Biosystems Model 380A Synthesizer. The sequence corresponded to nucleotides 108-146 of the published clone of Chambers and Keen (4). The probe was end-labelled with T_4 polynucleotide kinase and $Y^{32}P$ ATP for 30 min at 37°C in 50mM Tris HCl (pH7.4) 10mM $MgCl_2$,50mM DTT, 0.1mM ATP followed by removal of unincorporated label. Hybridisation was carried out in 25% formamide, 5xSSC (SSC is 0.15MNacl, 15mM sodium citrate pH7.0), 0.25% milk powder at 42°C. Washings were in 5 x SSC at 65°C.

Northern Blotting and DNA Sequencing

Northern blots using mRNA from the JOY cell line and K562 were performed basically as described by Hariharan (17) using a cDNA probe xtending from nucleotides 1-500 of our sequence. Control probings w re perform d with c-abl cDNA.

DNA sequencing was performed by th chain termination method in M13 vectors, with Klenow and γP^{32} dATP (18). The sequence was analysed using the computer programs of Staden (19).

Expression Systems and Epitope Mapping.

The pUR series of vectors (20) was used to express the entirety of the La cDNA and 3 fragments of it generated by digestion with ScaI. Bacterial lysates were subjected to SDS-polyacrylamide gel electrophoresis, electroblotted onto nitrocellulose (21), and probed with various autoimmune sera, followed by ^{125}I-labelled protein A and autoradiography. All blockings, washings and antibody probings were performed with 5% milk powder in phosphate buffered saline (22). Serum dilutions were 1 in 200 and the Protein A-I^{125} had a specific activity of 40 µCi/ml. Sera were absorbed with sonicates of various expressing clones to delete particular specificities.

Purifications of Recombinant La and its use in an ELISA.

The La cDNA was subcloned into a vector (pEV)(23) in which the recombinant protein is expressed as a fusion protein only with a few amino acids from the polylinker of the vector. The recombinant La was purified by the method described to purify native La from HeLa cells (9), and used to develop an ELISA (See Example 2).

Affinity Purified Anti La Antibodies.

Recombinant La was bound to CnBr activated Sepharose beads and reacted with the IgG portion of anti La containing sera. After extensive washing the bound anti La Ab was eluted with glycine HCl pH2.6 (25). Various control s ra were appli d to ensure

specificity. Th eluted, affinity purified anti La antibodies were tested using indirect immunofluorescence on HeLa cells and immunoblotting against a soluble nuclear preparation of HeLa cells (6). A murine monoclonal against La was also used to ensure the identity of the recombinant material (26).

RESULTS

Isolation and Characteristics of Human cDNA clone Encoding La.

A cDNA library constructed from the JOY cell line, a human B cell tumor line, was screened with the oligonucleotide probe and this detected approximately one hybridizing clone per 10,000 plaques. The largest of these, 1400 bases in length, was selected for further study. The sequence of this DNA (Fig.1) reveals a single open reading frame beginning with an authentic EcoRI site and continuing for 1065 coding bases (encoding a polypeptide of 355 amino acids), followed by 200 bases of 3' noncoding sequence and a polyA tail. Northern blot analysis, using mRNA derived from 2 human cell lines, the Raji cell (an EBV infected B cell) and JOY, demonstrates that the full length La mRNA is approximately 2kb, and that therefore the derived DNA sequence for La is deficient by 500-600 5' bases. The translation product has a predicted weight of 40.5kD, approximately 9kD less than maximum estimates of the MW of native La in reducing polyacrylamide gels. Analysis using the Kyte and Doolittle method revealed a relatively hydrophilic molecule, with no regions suggestive of membrane insertion.

A number of data banks were computer searched for homologies betw en La and previously

described DNA or protein sequences, however, no homologies with published sequences were found other than at the 5' end (the La sequence of Chambers and Keene (4)). Because it has been suggested that cross reactions between microbiological antigens and self proteins could be responsible for the development of some autoantibodies (27), particular attention was paid to possible homologies between regions of La and any sequenced viral or bacterial antigens. No such homologies were found.

Comparison of sequence with previously published partial sequence.

When the La sequence of Fig.1 is compared to that of Chambers and Keene (4), anomalies are apparent. In particular, a single cytidine residue present at position 134 in the present sequence is missing from the published clone 159 of the published sequence). This leads to a false stop codon being read at position 168 of the published sequence. No such stop codon is found in the present sequence. To verify the present sequence, this region has been sequenced, in both orientations, and 2 independent clones used, one isolated from the JOY library and one from a T cell tumor library. Accordingly, there is no doubt that the present sequence interpretation is correct. The predicted molecular weight of the La molecule encoded by the present cDNA is 40.1kD, which would be a minimum estimate not including post translational changes.

Expression Systems and ELISA.

When the La cDNA is subcloned into pEV (23), immunoreactive La of MW approximately 40kD is produced, in agreement with predicted MW from the DNA sequence. The evidence that this recombinant prot in

is La, comes from several experiments:- first, only sera shown by standard methods (counter immuno lectrophor sis against CDC prototype anti La or immunoblotting) to contain anti La will react with the recombinant protein; second, all standard anti La sera react with the recombinant protein; third, antibodies affinity purified on a column containing the recombinant protein display the immunofluorescent and immunoblot patterns characteristic of anti La; and fourth, a murine monoclonal directed to La reacts with the recombinant protein. Thus, there is overwhelming evidence that this cDNA encodes immunoreactive La.

Western blotting using the pEV produced La is an extremely sensitive method for the detection of anti La antibodies. Serum diluted to 1 in 10^5 produces strong signals against recombinant La. Recombinant La has been purified from pEV containing E.coli cultures using a method derived from Stefano (8), designed to purify La from mammalian cells. This is a simple method producing approximately 25 µgm recombinant La/1ml culture. (See Example 2).

Epitopes on the La molecule.

Published data (28) from experiments using partial enzymatic digestion of native La in mammalian cell extracts, suggests that 2 regions of the La molecule can be separately recognised by anti La sera. Some patient sera react with both regions in immunoblots while others react with one or the other. The previously published partial cDNA for La (4) predicted that one epitope might be the strongly hydrophilic decapeptide from amino acids 31-40. To investigate epitopes on La, 3 fragments of the present cDNA, g nerat d by ScaI digestion have been

subcloned and expressed separately in plasmid vectors. One fragment was reactive with anti-La s ra, establishing the existence of at least one epitope. Subclone LaI (bases 1-251) was subcloned into BTA224 and expressed as a stable fusion protein with β-galactosidase. This portion of the La molecule is not immunoreactive with anti La sera by colony immunoassay or Western blotting. Subclone LaII (bases 252-755) was also subcloned into BTA224 and an unstable fusion protein produced which has no detectable reactivity with human antibodies. Subclone LaIII (bases 756-1065) was subcloned using Bam linkers, into the pUR291 vector, and produced a stable fusion protein which is strongly immunoreactive in the colony immunoassay and in Western blots. In order to determine if the present cDNA encodes all the epitopes of native La, absorption experiments have been carried out. Anti La sera, absorbed with sonicates of E.coli expressing the recombinant La molecule, still demonstrate strong reactivity to the 50kD La band in a Western blot using a nuclear preparation of HeLa cells.

EXAMPLE 2

This Example illustrates the expression of recombinant La in cell culture, purification of the product from the culture medium, and use of the purified product in a diagnostic assay.

In the drawings:

Figure 2 shows: (a) demonstration by immunofluorescence of reactive between nuclei of HEp 2 cells and serum antibody affinity purified using recombinant La.

(b) Western blot with cloned La and native La. Lanes 1 and 5 illustrate reactivity with affinity-purified human anti-La using recombinant La; lanes 2 and 6 reactivity with the murine monoclonal anti-La, SW5(26); lanes 3 and 7 reactivity with human anti-La; and lanes 4 and 8 non-reactivity with serum from a healthy subject.

Figure 3 shows ELISA units of anti-La in sera of patients with various autoimmune diseases and healthy subjects tested against the recombinant La nucleoprotein.

MATERIALS AND METHODS

Sera

Coded sera from 260 patients with various autoimmune diseases associated with antinuclear antibodies (ANA) and from 100 normal subjects were tested for anti-La by ELISA using recombinant La. The disease groups included primary Sjögren's syndrome (50 patients), secondary Sjögren's syndrome associated with rheumatoid arthritis (14 patients), rheumatoid arthritis (RA(20 patients)), systemic lupus erythematosus (SLE(50 patients)), mixed connective tissue disease (MCTD(19 patients)), scleroderma (50 patients), primary biliary cirrhosis (PBC(18 patients)) and autoimmune chronic active hepatitis (A-CAH(39 patients)). All patients except those with secondary Sjögren's syndrome associated with rheumatoid arthritis were considered to have one disease only except one patient with primary Sjögren's syndrome who had had acute classical SLE 26 years previously (20).

Purification of cloned La

E.coli stock contained a pEV vector (23[illegible], with a 1.4kb cDNA for La as described abov was grown

in liquid culture. Recombinant La production was induced by heat and the E.coli harvested by centrifugation. The bacterial pellet was suspended in Buffer 1 (10mM Tris-HCl pH 8, 2mM EDTA, 50mM NaCl) with 0.2mgs/ml lysozyme and mixed for 30 minutes at room temperature prior to addition of a final 0.2% concentration of Triton X-100. After mixing for 10 minutes an equal volume of Buffer 1 containing 10mM $MgCl_2$, was added with 2µg/ml DNAse (Cooper Biomedical, Malvern, Pennsylvania) and the suspension mixed for a further 15 minutes. 5µg/ml of the protein inhibitors, leupeptin, pepstatin A, chymostatin (Sigma, St.Louis) and 0.5mM PMSF (Calbiochem, California) were added and mixed for 5 minutes. The mixture was centrifuged at 2,000 rpm for 5 minutes and the resulting supernate centrifuged at 18,000 rpm f : 30 minutes at 4°C. The recombinant La protein was then purified according to a method described by Stefano (8) for purification of native La nucleoprotein from HeLa cells. In preliminary studies all steps in the purification wer monitored by Western blotting using a high titre anti-La-positive serum, and the results were compared with those obtained in the same Western blot using a nuclear-enriched extract of HeLa cells (14). The procedure resulted in the isolation of two immunoreactive polypeptides which were pooled and dialysed. The yield of protein was measured by the Lowry assay and the stability of the La preparation was assessed after storage under various conditions: at 4°C in solution, at -70°C in solution, after lyphilisation, and coated on to plastic microtitration plates (Flow Laboratories, Virgina, USA).

ELISA

Flat-bottomed wells of a 96 well microtitration plate were coated overnight at 4°C with 1µg lyophilised recombinant La in 50µL distilled water. Uncoated sites in the wells were blocked by 100µL 10% fetal calf serum in phosphate-buffered saline pH 7.4 (PBS) for one hour at room temperature. The plate was washed in PBS, triplicates of 50µL sera diluted 10^{-3} were applied for 3 hours at room temperature and the plate was again washed. Goat anti-human globulin conjugated to alkaline phosphatase (Sigma, St.Louis, USA) was applied for 90 minutes at room temperature, followed by disodium p-nitrophenyl phosphate substrate solution (Sigma, St.Louis, USA). Chromophore development was monitored by measuring absorbance at 405nm before and after addition of 3M NaOH using a Titertek Multiskan ELISA reader. For each assay a standard curve was constructed using 10-fold dilutions of the same high titre anti-La-positive serum. One unit of binding activity was defined as that given by the 10^{-6} dilution of the anti-La-positive control serum. Those sera with binding activities of (5 units at 10^{-3} dilution were arbitrarily defined as positive; this was based on an optical absorbance (5 standard deviations above the mean value for 50 normal subjects.

RESULTS

Two polypeptides, a less abundant one of 37kD and a major one of 30kD, were obtained after purification of the cultures of E.coli containing the expression vector with the 1.4 kb insert of cDNA for La. The average yield of the recombinant La protein was 25µg/ml culture. Proof that the product from

E.coli was the La nucleoprotein was obtained as follows. Human anti-La that was affinity purified from recombinant La on a cyanogen bromide-activated Sepharose column (Pharmacia, Sweden) reacted with nuclei of HEp2 cells giving the characteristic La ANA pattern by immunofluorescence and also reacted with native La by Western blotting (Fig.2a and 2b); a La-specific murine monoclonal antibody, SW5, kindly provided by Dr.David Williams, Kennedy Institute, London, reacted with the recombinant La; and reactivity between human anti-La and native La was inhibited by recombinant La but not by a recombinant protein from the same strain of E.coli containing the pEV vector with a different cDNA. The preparation of recombinant La was stable for at least two months when stored in solution at 4°C and at -70°C, after lyophilisation at -70°C, and on microtitration plates stored at 4°C.

Antibodies to recombinant La protein were detected by ELISA in 47 (94%) of 50 patients with primary Sjögren's syndrome, in 1 (7%) of 14 patients with secondary Sjögren's syndrome but in none of 20 patients with RA, 50 patients with SLE, 19 patients with MCTD, 50 patients with scleroderma, 18 patients with PBC, 39 patients with A-CAH, nor in 100 healthy subjects (Fig.3). Sera from all 48 patients who were anti-La-positive by ELISA reacted at a dilution of 10^{-3} or greater with both recombinant La and native La by Western blotting.

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CLAIMS:

1. A diagnostic test for the detection of the antinuclear antibody anti-La in a serum sample, which comprises the steps of:

(a) contacting the serum sample with a support having immobilised thereon a synthetic polypeptide displaying the antigenicity of all or a portion of the human autoantigen La or an antigenically active fragment thereof, the amino acid sequence of said polypeptide or fragment comprising or including the sequence

LeuGluGlyGluValGluLysGluAlaLeuLysLysIleIle
GluAspGlnGlnGluSerLeuAsnLysTrpLysSerLysGly
ArgArgPheLysGlyLysGlyLysGlyAsnLysAlaAlaGln
ProGlySerGlyLysGlyLysValGlnPheGlnGlyLysLys
ThrLysPheAlaSerAspAspGluHisAspGluHisAspGlu
AsnGlyAlaThrGlyProValLysArgAlaArgGluGluThr
AspLysGluGluProAlaSerLysGlnGlnLysThrGluAsn
GlyAlaGlyAspGln

or an epitope thereof; and

(b) detecting the presence of anti-La antibody in said serum bound to said synthetic polypeptide or fragment.

2. A diagnostic test according to claim 1, wherein said synthetic polypeptide comprises an amino acid sequence substantially as set out in Figure 1.

3. A diagnostic test kit according to claim 1 or claim 2, wher in said synthetic polypeptide comprises a fused polypeptide produced by expression of a recombinant DNA molecule in a host cell.

4. A diagnostic test according to claim 1, wherein said step (b) comprises addition of an enzyme-linked anti-human globulin antiserum, followed by addition of an appropriate substrate for said enzyme and detection or determination of the action on said substrate enzyme bound to the support.

5. A diagnostic test kit for detection of antinuclear antibody anti-La in a serum sample, which comprises:

(a) a support having immobilised thereon a synthetic polypeptide displaying the antigenicity of all or a portion of the human autoantigen La or an antigenically active fragment thereof, the amino acid sequence of said polypeptide or fragment comprising or including the sequence

LeuGluGlyGluValGluLysGluAlaLeuLysLysIleIle
GluAspGlnGlnGluSerLeuAsnLysTrpLysSerLysGly
ArgArgPheLysGlyLysGlyLysGlyAsnLysAlaAlaGln
ProGlySerGlyLysGlyLysValGlnPheGlnGlyLysLys
ThrLysPheAlaSerAspAspGluHisAspGluHisAspGlu
AsnGlyAlaThrGlyProValLysArgAlaArgGluGluThr
AspLysGluGluProAlaSerLysGlnGlnLysThrGluAsn
GlyAlaGlyAspGln

or an epitope thereof; and

(b) means for detecting the presenc of anti-La antibody in said serum bound to said synthetic polypeptide or fragment.

6. A diagnostic test kit according to claim 4, wherein said synthetic polypeptide comprises an amino acid sequence substantially as set out in Figure 1.

7. A diagnostic test kit according to claim 5 or claim 6, wherein said synthetic polypeptide comprises a fused polypeptide produced by expression of a recombinant DNA molecule in a host cell.

8. A diagnostic test kit according to claim 5, wherein said means for detecting the presence of bound anti-La antibody comprises enzyme-linked anti-human globulin antiserum and an appropriate substrate for said enzyme.

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GluPheAsnArgLeuAsnArgLeuThrThrAspPheAsnValIleValGluAlaLeuSerLysSerLysAlaGluLeuMetGlu
GAATTCAACAGGTTGAACCGTCTAACAACAGACTTTAATGTAATTGTGGAAGCATTGAGCAAATCCAAGGCAGAACTCATGGAA
        10        20        30        40        50        60        70        80
IleSerGluAspLysThrLysIleArgArgSerProSerLysProLeuProGluValThrAspGluTyrLysAsnAspVal.Lys
ATCAGTGAAGATAAAACTAAAATCAGAAGGTCTCCAAGCAAACCCCTACCTGAAGTGACTGATGAGTATAAAAATGATGTAAAA
        94       104       114       124       134       144       154       164
AsnArgSerValTyrIleLysGlyPheProThrAspAlaThrLeuAspAspIleLysGluTrpLeuGluAspLysGlyGlnVal
AACAGATCTGTTTATATTAAAGGCTTCCCAACTGATGCAACTCTTGATGACATAAAAGAATGGTTAGAAGATAAAGGTCAAGTA
       178       188       198       208       218       228       238       248
LeuAsnIleGlnMetArgArgThrLeuHisLysAlaPheLysGlySerIlePheValValPheAspSerIleGluSerAlaLys
CTAAATATTCAGATGAGAAGAACATTGCATAAAGCATTTAAGGGATCAATTTTTGTTGTGTTTGATAGCATTGAATCTGCTAAG
       262       272       282       292       302       312       322       332
LysPheValGluThrProGlyGlnLysTyrLysGluThrAspLeuLeuIleLeuPheLysAspAspTyrPheAlaLysLysAsn
AAA.TTGTAGAGACCCCTGGCCAGAAGTACAAAGAAACAGACCTTCTAATACTTTTCAAGGACGATTACTTTGCCAAAAAAAAT
       346       356       366       376       386       396       406       416
GluGluArgLysGlnAsnLysValGluAlaLysLeuArgAlaLysGlnGluGlnGluAlaLysGlnLysLeuGluGluAspAla
GAAGAAAGAAAACAAAATAAAGTGGAAGCTAAATTAAGAGCTAAACAGGAGCAAGAAGCAAAACAAAAGTTAGAAGAAGATGCT
       430       440       450       460       470       480       490       500
GluMetLysSerLeuGluGluLysIleGlyCysLeuLeuLysPheSerGlyAspLeuAspAspGlnThrCysArgGluAspLeu
GAAATGAAATCTCTAGAAGAAAAGATTGGATGCTTGCTGAAATTTTCGGGTGATTTAGATGATCAGACCTGTAGAGAAGATTTA
       514       524       534       544       554       564       574       584
HisIleLeuPheSerAsnHisGlyGluIleLysTrpIleAspPheValArgGlyAlaLysGluGlyIleIleLeuPheLysGlu
CACATACTTTTCTCAAATCATGGTGAAATAAAATGGATAGACTTCGTCAGAGGAGCAAAAGAGGGGATAATTCTATTTAAAGAA
       598       608       618       628       638       648       658       668
LysAlaLysGluAlaLeuGlyLysAlaLysValAlaAsnAsnGlyAsnLeuGlnLeuArgAsnLysGluValThrTrpGluVal
AAAGCCAAGGAAGCATTGGGTAAAGCCAAAGTTGCAAATAATGGTAACCTACAATTAAGGAACAAAGAAGTGACTTGGGAAGTA
       682       692       702       712       722       732       742       752
LeuGluGlyGluValGluLysGluAlaLeuLysLysIleIleGluAspGlnGlnGluSerLeuAsnLysTrpLysSerLysGly
CTAGAAGGAGAGGTGGAAAAAGAAGCACTGAAGAAAATAATAGAAGACCAACAAGAATCCCTAAACAAATGGAAGTCAAAAGGT
       766       776       786       796       806       816       826       836
ArgArgPheLysGlyLysGlyLysGlyAsnLysAlaAlaGlnProGlySerGlyLysGlyLysValGlnPheGlnGlyLysLys
CGTAGATTTAAAGGAAAAGGAAAGGGTAATAAAGCTGCCCAGCCTGGGTCTGGTAAAGGAAAAGTACAGTTTCAGGGCAAGAAA
       850       860       870       880       890       900       910       920
ThrLysPheAlaSerAspAspGluHisAspGluHisAspGluAsnGlyAlaThrGlyProValLysArgAlaArgGluGluThr
ACGAAATTTGCTAGTGATGATGAACATGATGAACATGATGAAAATGGTGCAACTGGACCTGTGAAAAGAGCAAGAGAAGAAACA
       934       944       954       964       974       984       994      1004
AspLysGluGluProAlaSerLysGlnGlnLysThrGluAsnGlyAlaGlyAspGln***
GACAAAGAAGAACCTGCATCCAAACAACAGAAAACAGAAAATGGTGCTGGAGACCAGTAGTTTAGTAAACCAATTTTTTATTCA
      1018      1028      1038      1048      1058      1068      1078      1088
TTTTAATAGGTTTTAACGACTTTTGTTTGCGGGGCTTTTAAAAGGAAAACCGAATTAGGTCCACTTCATGTCCACCTGTGAGAA
      1102      1112      1122      1132      1142      1152      1162      1172
AGGAAAATTTTT
      1186
```

FIGURE 1

FIGURE 2a

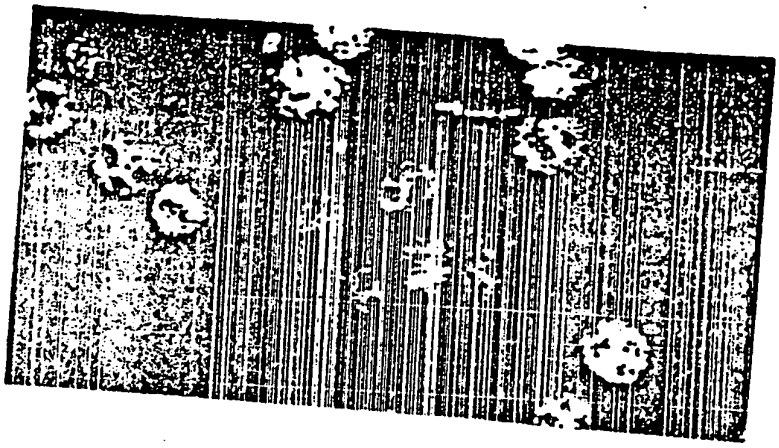


FIGURE 2b

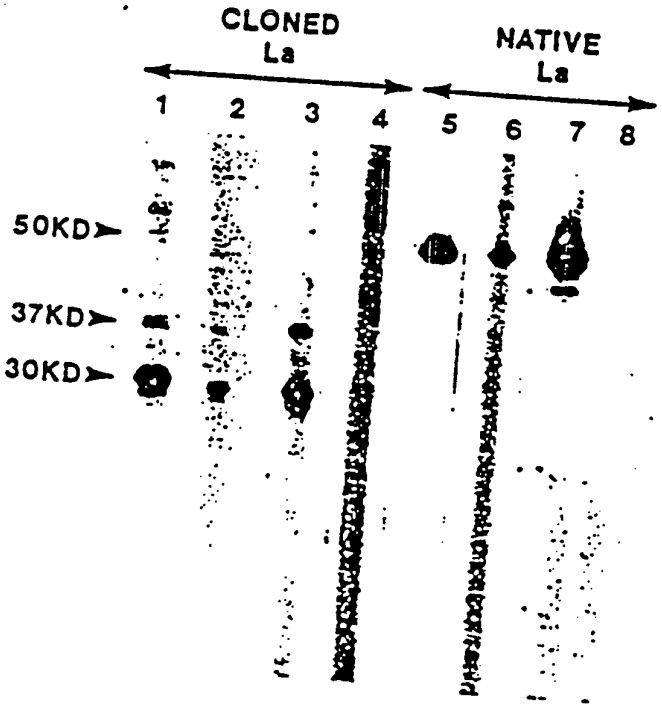
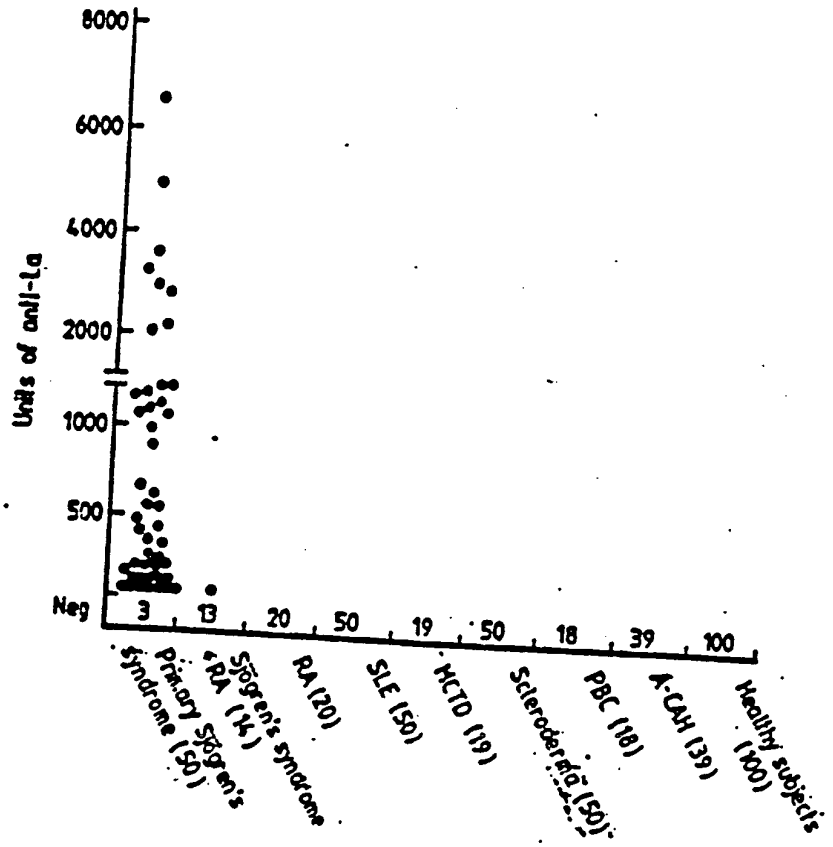


FIGURE 3


8000
6000
4000
2000
1000
500
Neg
Units of anti-La
3
13
20
50
19
50
18
39
100
Primary Sjögren's syndrome (50)
Sjögren's syndrome +RA (14)
RA (20)
SLE (50)
MCTD (19)
Scleroderma (50)
PBC (18)
A-CAH (39)
Healthy subjects (100)

INTERNATIONAL SEARCH REPORT

International Application No PCT/AU88/00177

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[4] G01N 33/53, 33/543, 33/553, 33/564, C12N 15/00

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System	Classification Symbols
IPC	G01N 33/53, 33/543, 33/533, 33/564

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched

AU: IPC AS ABOVE

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	WO,A, 83/00877 (ICL SCIENTIFIC) 17 March 1983 (17.03.83)	1
A	EP,A, 206779 (MODERN DIAGNOSTICS INC) 30 December 1986 (30.12.86)	1
A	US,A, 4727137 (VALLEE ET AL) 23 February 1988 (23.02.88)	1
A	US,A, 4314987 (MORRIS ET AL) 9 February 1982 (09.02.82)	1

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1 AUGUST 1988 (01.08.88)	10 AUGUST 1988 (10.08.88)
International Searching Authority	Signature of Authorized Officer
AUSTRALIAN PATENT OFFICE	JOHN G. HANSON

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 88/00177

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members		
WO 8300877	AU 89546/82	CA 1206092	EP 91911
	ES 515350	ES 524183	
EP 206779	JP 62032363		
US 4727137	DK 6056/86	EP 220241	WO 8606079

END OF ANNEX